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Broadly cross-reactive HIV neutralizing human monoclonal antibody Fab selected by sequential antigen panning of a phage display library

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Abstract

Identification of broadly cross-reactive human monoclonal antibodies (mAbs) has major implications for development of vaccines, inhibitors and research tools. Here we describe a sequential antigen panning (SAP) methodology that may facilitate the selection of such antibodies. An HIV-specific antibody Fab (m18) was selected from a human Fab phage-display library by SAP against several recombinant soluble HIV envelope glycoproteins (Envs) and Env–sCD4 complexes. This Fab bound to a variety of recombinant soluble Envs (gp140s) from primary HIV isolates representing different clades, and inhibited cell fusion and virus entry mediated by Envs of primary HIV isolates. The methodology and the results may have implications for development of HIV vaccines and inhibitors, as well as for identification of antibodies to conserved epitopes on rapidly mutating viruses and cells.
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1. Introduction

A fundamental problem in prevention and treatment of HIV infections is the virus ability to rapidly generate mutants resistant to immune responses and drugs. Identification and characterization of conserved

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HIV structures may therefore help to solve this problem. Although HIV has evolved a number of strategies to evade immune responses to conserved structures and cross-reactive neutralizing antibody response is generally weak, broadly cross-reactive HIV neutralizing human monoclonal antibodies (nhmAbs) do exist. Finding immunogens that are able to elicit them could be facilitated by the exploration of the interaction of these antibodies with the HIV envelope glycoproteins (Envs)—an approach known as "retrovaccinology" (Burton, 2002). However, only few broadly cross-reactive HIV nhmAbs have been identified to date and efforts to use mimetics of their epitopes or portions of the epitopes as immunogens are ongoing but have produced only limited success so far (Zwick et al., 2001a). The identification of new broadly cross-reactive HIV nhmAbs and their conserved epitopes is therefore of obvious importance for the development of effective HIV vaccines and drugs.

We hypothesized that identification and selection of high-affinity antibodies against conserved epitopes can be facilitated by sequentially changing the antigen during the panning of phage display libraries. Here we describe a new methodology for efficient selection of broadly cross-reactive antibodies termed sequential antigen panning (SAP) and report the identification of an HIV nhmAb designated m18. This antibody was selected from a human Fab phage display library using SAP against $gp140_{89.6}$–sCD4, $gp140_{IIIB}$–sCD4, $gp140_{89.6}$ and $gp140_{IIIB}$ followed by screening with $gp140_{89.6}$, $gp120_{JR\text{-}FL}$ and $gp140_{IIIB}$, and their complexes with soluble CD4 (sCD4). The antibody bound to Envs from different HIV isolates with high affinity, and potently inhibited HIV entry and membrane fusion mediated by Envs of primary HIV isolates from a variety of clades.

2. Materials and methods

2.1. Cells, viruses, plasmids, soluble CD4 (sCD4), gp120, gp140 and antibodies

293T cells were purchased from ATCC. The CEM cells expressing CCR5 (CEM-CCR5) were a gift from J. Moore (Cornell University, New York, NY). Recombinant vaccinia viruses used for the fusion assay were described previously (Nussbaum et al., 1994). Plasmids used for expression of various Envs were obtained through the NIH AIDS Research and Reference Reagent Program (ARRRP) from B. Hahn (University of Alabama at Birmingham). Two-domain soluble CD4 (sCD4) was obtained from the ARRRP. $Gp120_{89.6}$ and $gp140_{89.6}$ were produced by recombinant vaccinia viruses provided by R. Doms, University of Pennsylvania, Philadelphia, PA. $Gp120_{IIIB}$, $gp140_{IIIB}$, and a battery of gp140s derived from HIV-1 primary isolates were also prepared from recombinant vaccinia viruses; these were: 92UG037.8 (clade A), 92HT593.1 (clade B), 93MW965.26 (clade C), 93ZR001.3 and 92UG024.2 (clade D), 92TH022.4 (clade E), CM243 (clade E), and R2 (clade B). All recombinant envelope glycoproteins were purified by a combination of lentil lectin affinity chromatography using Sepharose 4B (Pierce, Rockford, IL) followed by size exclusion chromatography using HiLoad 16/60 Superdex 200 prep grade column (Amersham Pharmacia Biotech, Piscataway, NJ). Recombinant $gp120_{JR\text{-}FL}$ was provided by A. Schultz and N. Miller (NIAID, Bethesda, MD). The human monoclonal antibody X5 was produced as described (Moulard et al., 2002), and the following antibodies were purchased: polyclonal sheep anti-gp120 antibody D7324 (Sigma), HRP conjugated monoclonal mouse anti-M13 antibody (Pharmacia, Uppsala, Sweden) and HRP conjugated polyclonal anti-human IgG F(ab′)2 antibodies (Jackson ImmunoResearch, Westgrove, PA). The human monoclonal antibody 17b was a gift from J. Robinson (Tulane University Medical Center, New Orleans, LA) and b12-from D. Burton (The Scripps Research Institute, La Jolla, CA).

2.2. Sequential antigen panning (SAP) of phage library and analysis of selected phage clones

pComb3H library was constructed using pComb3H phagemid vector and 30 cm^3 of bone marrow obtained from three long-term nonprogressors whose sera exhibited the broadest and most potent HIV-1 neutralization (A, H and K) among 37 HIV-infected individuals (T. Evans et al., in preparation). Phage (5×10^{12} cfu/ml) were preadsorbed on streptavidin-M280-Dynabeads in PBS for 1 h at room temperature (RT) followed by depletion in an immunotube (Nunc, Denmark) coated with 10 μg/ml sCD4 for 1 h at 37 °C.

Depleted phage library was incubated with 50 nM biotinylated HIV-1 envelope glycoprotein $gp140_{89.6}$ complexed with sCD4 in solution ($gp140_{89.6}$/sCD4=1:1 molar ratio) for 2 h at RT with gentle agitation. Phage binding to biotinylated envelope glycoprotein was separated from the phage library using streptavidin-M280-Dynabeads and a magnetic separator (Dynal). The beads were washed 20 times with 1 ml of PBS containing 0.1% Tween-20 and another 20 times with 1 ml of PBS. Bound phage were eluted by incubation at RT for 10 min with 1 ml of 100 mM Triethanolamine followed by neutralization with 0.5 ml of 1 M, pH 7.5 Tris–HCl. Eluted phage were rescued by infection of *E. coli* TG1 cells and phage library was prepared for the next round of panning. For the 2nd round of panning, the phage library was preadsorbed on streptavidin-M280-Dynabeads and immobilized sCD4 as before and 50 nM of biotinylated $gp140_{IIIB}$ complexed with sCD4 (1:1 on molar level) used as antigen. For the 3rd and 4th rounds of panning, 10 nM (2 nM for 5th and 6th rounds) of biotinylated $gp140_{89.6}$ and $gp140_{IIIB}$ alone were sequentially used as antigens. For each library, 20 individual clones after the 4th, 5th and 6th round of panning were screened by phage ELISA for binding to $gp140_{89.6}$, $gp120_{JRFL}$, and $gp140_{IIIB}$, and their complexes with sCD4 as follows. Single colonies were inoculated into 1 ml of 2× YT medium containing 100 μg/ml ampicillin and 2% glucose in 12-ml falcon tubes. The tubes were incubated overnight at 37 °C/250 rpm. Ten microliters of overnight culture from each tube was inoculated into 1 ml of 2× YT medium containing 100 μg/ml ampicillin, 2% glucose and about 4×10^9 cfu/ml of $M_{13}KO_7$ in 12-ml falcon tubes. The phage tubes were incubated at 37 °C/250 rpm for 2 h and centrifuged at 4000 rpm for 10 min at RT. The supernatant was removed and the cells were suspended in 1 ml of 2× YT medium with 100 μg/ml ampicillin and 50 μg/ml kanamycin. The tubes were then incubated overnight at 30 °C/250 rpm. After 16 h, the tubes were centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was used for phage ELISA.

2.3. Phage ELISA

ELISA was performed by using 96-well Nunc-Immuno™ Maxisorp™ surface plates (Nalge Nunc International) which were coated overnight at 4 °C with 100 μl of gp120/140 (1 μg/ml in sodium bicarbonate buffer, pH 8.3) or gp120/140–sCD4 complex (100 μg/ml gp120/140 in PBS was premixed with equal volume of 100 μg/ml sCD4. After incubation at RT for 30 min, the mixture was diluted to 1 μg/ml in PBS, blocked in 100 μl of 4% non-fat dry milk in PBS) for 1 h at 37 °C. After four washes with 0.05% Tween-20/PBS washing buffer (WB), wells were incubated with 100 μl of phage supernatant for 2 h at 37 °C. Bound phage were detected by using horse radish peroxidase (HRP) labeled anti-M13 monoclonal antibody (Pharmacia) with incubation for 1 h at 37 °C and revealed by adding ready-to-use ABTs substrate (Pharmacia). Color development was performed at RT for 15 min and monitored at 405 nm.

2.4. Preparation of soluble Fab fragment

Phagemid DNA from the selected clone was prepared and digested with *Sac*I and *Spe*I. The fragment band was extracted and purified from agarose gel and ligated with phagemid pComb3X digested with the same enzymes. Ligated product was transformed to *E. coli* Top 10. pComb3X phagemid vector has amber stop codon between Fab insert and gIII, which allows expression of soluble Fab in a non-suppressor bacteria strain, like Top 10. Soluble Fab was produced as described (Barbas et al., 2001). Protein G columns were used for purification.

2.5. Binding of soluble Fab

ELISA was performed by using 96-well Nunc-Immuno™ Maxisorp™ surface plates. Coating of antigen and washing and blocking steps were the same as described in phage ELISA. Microplate wells were incubated with 100 μl two-fold serially diluted soluble Fab for 2 h at 37 °C. After four washes with WB, 100 μl of a 1:2500 dilution of HRP-conjugated goat anti-human IgG $F(ab')_2$ was added and incubated for 1 h at 37 °C. Following four washes with WB, the assay was developed at 37 °C for 15–30 min with ready-to-use ABTs substrate and monitored at 405 nm. For capture ELISA, 1 μg/ml polyclonal sheep anti-gp120 antibody D7324 was coated on the microplate to capture Envs. Then the Env was added and the steps described above were followed.

2.6. Cloning of HIV-1 envelope genes

Viruses pseudotyped with envelopes from HIV-1 primary viruses representing HIV-1 group M, clades A–F, and one laboratory adapted HIV-1 isolates were used in this study. PBMCs from patients infected with HIV-1 subtype B (VI1399, VI1273, VI423) and HIV-1 subtype F (14004) were obtained from the Institute of Tropical Medicine, Antwerp, Belgium. The sera of patients VI423 and 14004 have been observed to posses broadly cross-reactive neutralizing antibody responses (Beirnaert et al., 2000). The R2 (clade B) envelope (AD001) was from a patient whose sera demonstrated broad cross-neutralizing activity previously cloned and has been described elsewhere (Quinnan et al., 1999). Isolates GX-C44 (clade C) and GX-E14 (clade E) were obtained from the National Center for AIDS/STD Control and Prevention, Beijing, China. The molecular virus clones of TH966, Z2Z6 and 9RW020.5 were obtained from the National Institute of Health (NIH) AIDS Research and Reference Reagent Program (ARRRP). The primary MN (MN-P) and the T-cell line adapted (TCLA) clones, P37 and V5 have been described (Park et al., 1998). Finally, primary virus envelope clones #6-4/41, #8-4/49, #4-4/116, and #9-131 were obtained from the Multicenter AIDS Cohort Study (MACS) and have been described elsewhere (Quinnan et al., 1998). HIV-1 envelope genes were cloned by RT-PCR or nested PCR using the rTth DNA polymerase (Applied Biosystems, Foster City, CA). The envelope genes were cloned into the pSV7d expression vector (Chiron) for all patients with the exception of the 92RW020.5 clone, which was in the pSVIII expression vector. Nucleotide sequencing was performed on envelope clones using the dideoxy cycle sequencing technique. Sequencing was done using a V3 specific primer primarily to confirm identity of gene clones not previously described.

2.7. Pseudovirus preparation

Pseudotype viruses were prepared by cotransfection of 70% to 80%—confluent 293T cells with pNL4-3.luc.E-R—(NIH ARRRP) and pSV7d-env plasmid using the calcium phosphate/HEPES buffer technique, according to manufacturers instruction (Promega). Sixteen hours after the transfection, the media was removed and replaced with media supplemented with 0.1 mM sodium butyrate (Sigma). Cells were allowed to grow for an additional 24 h. The supernatant was harvested, centrifuged at 16,000 rpm for 5 min at 4 °C, filtered through a 0.45-μm pore filter and either used fresh or kept frozen at −80 °C.

2.8. Infectivity and neutralization assays

Infectivity and neutralization assays were carried out using HOS $CD4^+$ $CCR5^+$, or HOS $CD4^+$ $CXCR4^+$ cells, as appropriate. Infectivity titers were determined on the basis of luminescence measurements at 3 days post-infection of the cells by the pseudotyped viruses. Neutralization assays were carried out in triplicate wells by preincubation of serial dilutions of the antibodies with pseudotype viruses for 1 h at 4 °C followed by infection of $1–2\times10^4$ HOS $CD4^+$ $CCR5^+$ cells. Luminesence was measured after 3 days. The mean luminescence readings for triplicate wells were determined, and the endpoint was considered to be the last dilution of antibody at which the mean results from the test samples were less than 50% of the nonneutralized control mean. Neutralization assays for each envelope clone against the tested antibodies were carried out two or three times.

2.9. Cell–cell fusion assays

The cell–cell fusion assay was performed as previously described (Nussbaum et al., 1994; Moulard et al., 2002). Briefly, transfected with plasmids encoding various HIV Envs under the control of T7 promoter were infected with recombinant vaccinia virus encoding the gene for the T7 polymerase (vTF7.3) at a multiplicity of infection (MOI) 10, and mixed with target cells infected with recombinant vaccinia virus encoding the β-galactosidase gene. The β-gal fusion assay was performed 2 h after mixing the cells. The extent of fusion was quantitated colorimetrically. The inhibitory effect of the antibodies was evaluated by mixing them with the effector cells for 30 min at 37 °C and then performing the fusion assay as described above. The syncytia assay was performed by counting the

number of syncytia 4 h after mixing the cells as previously described (Dimitrov et al., 1991).

3. Results

3.1. Selection of a phage Fab (m18) by SAP

We hypothesized that by sequentially changing antigens during panning of phage display libraries and screening the panned libraries using different antigens, the selected phage will display Fabs against conserved epitopes shared among all antigens used during the entire selection process. Complexes of two different recombinant soluble Envs ($gp140_{89.6}$ and $gp140_{IIIB}$) with two-domain soluble CD4 (sCD4) and the Envs alone were used as antigens for phage library panning as described in the Materials and methods. After four, five and six rounds of panning, screening of individual phage clones was performed in phage ELISA with $gp140_{89.6}$, $gp120_{JR\text{-}FL}$ and $gp140_{IIIB}$, and their complexes with sCD4. Three clones were selected based on their significant binding to all six antigens used for screening including sCD4–gp120 and gp120 from an HIV isolate (JR-FL) which was not used for panning and has significant sequence differences compared to 89.6 and IIIB. One phage clone, designated m18, with the highest level of binding to all antigens was selected for further characterization. Phagemid DNA of m18 was prepared and sequenced. In a control experiment to access the efficiency of the SAP methodology, the panning was performed only with one antigen (sCD4–$gp140_{89.6}$). In this case, none of the clones tested bound to $gp140_{IIIB}$, sCD4–$gp140_{IIIB}$, $gp120_{JR\text{-}FL}$ and sCD4–$gp120_{JR\text{-}FL}$ (data not shown).

3.2. Binding of Fab m18 to gp120 and gp140 from different isolates

M18 bound to gp120 from the primary isolates 89.6 and JR-FL with an affinity (equilibrium dissociation constant) of 1 nM and to the TCLA strain IIIB with 0.1 nM as measured by an ELISA assay (Fig. 1). It also bound to Envs (gp140s) from primary isolates representing different clades (Fig. 2) with nM affinity to most of them (Table 1). The m18 binding was not significantly affected by sCD4 (Fig. 1 and data not

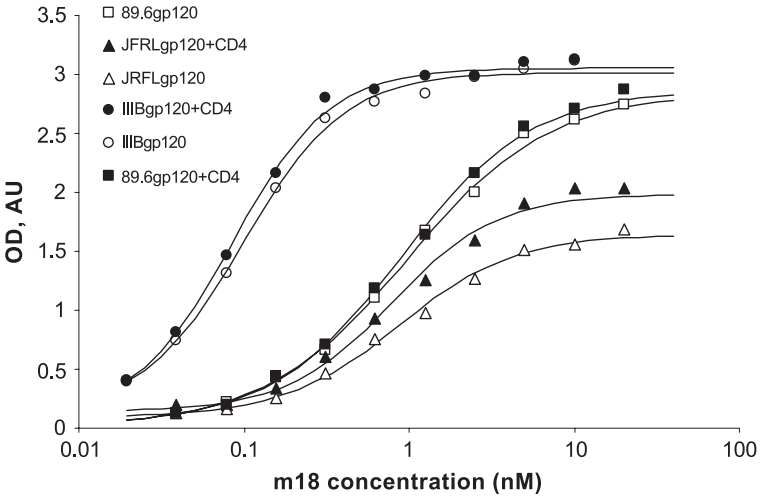

Fig. 1. Binding of m18 to gp120 and sCD4–gp120. Gp120s and gp120–sCD4 complexes were coated directly on 96-well plates, washed and m18 added at different concentrations. Bound Fabs were detected by anti-human IgG $F(ab')_2$-HRP and measured as optical densities at 405 nm. The background was estimated by the amount of Fabs bound to BSA and subtracted. The data were fitted to the Langmuir adsorption isotherm: $B/B_{max}=F/(K_d+F)$, where B is the amount of bound Fab, B_{max} is the maximal amount of bound Fab, F is its bulk concentration and K_d is the equilibrium dissociation constant. The continuous lines represent the data fitting and the symbols—the data. The values of K_d, which approximately correspond to concentrations requires for half-maximal binding (EC_{50}), are 1.0(0.9), 0.9(0.7) and 0.08(0.09) nM for 89.6, JR-FL and IIIB, respectively; in parentheses are the values for the sCD4–gp120 complexes.

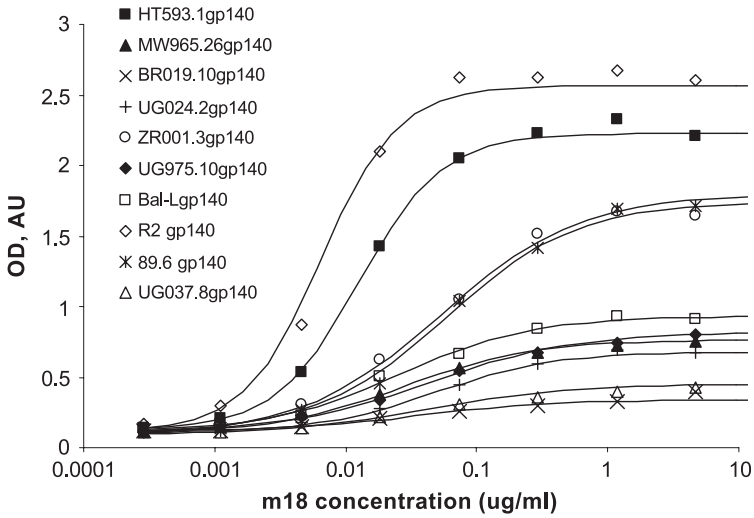

Fig. 2. Binding of m18 to gp140 from primary isolates. Gp140s were captured by the polyclonal sheep anti-gp120 antibody D7324 (5 μg/ml) coated on 96-well plates, washed and m18, was added at the indicated concentrations. Bound antibodies were detected by anti-human IgG F(ab′)$_2$-HRP and measured as optical densities at 405 nm. The data were fitted as described above. The K_d values (approximately equal to EC_{50}) of m18 for gp140 from primary isolates are summarized in Table 1.

shown). These results suggest that m18 is a broadly cross-reactive antibody Fab and its epitope is not CD4-induced.

3.3. Inhibition of cell fusion and virus entry by m18

To determine the breadth and potency of HIV-1 neutralization by m18, we measured its ability to inhibit cell fusion and virus entry mediated by Envs of primary isolates from different clades. In a cell–cell fusion assay, m18 inhibited various isolates with potency comparable but for some isolates slightly lower than the inhibitory activity of Fab X5 and Fab b12 (Table 2). Similar results were obtained by counting syncytia (data not shown). In a pseudotype virus assay, m18 inhibited entry to different extent of most of the isolates at concentrations up to 100 μg/ml; for 11 of the 15 primary isolates tested, the 50% neutralization was achieved at concentrations smaller than or equal to 0.1 mg/ml (Table 3). For comparison, the potent broadly HIV neutralizing human monoclonal antibody Fab X5 neutralized 13 out of 15 primary isolates with IC_{50} smaller than or equal to 0.1 mg/ml although on average, the X5 Ic_{50} were somewhat lower than those for m18. One should note that m18 is a Fab fragment, and because it is not CD4i antibody, one can expect that its neutralizing activity could be even higher as a whole antibody; it was recently demonstrated that IgG1 X5 is less potent than Fab X5 likely due to the fact that its epitope is induced by CD4 (CD4i antibody) and it exerts its neutralizing effect after the HIV binding to CD4 (Labrijn

Table 1
Binding of m18 to gp140 from primary isolates

Envelope gp140	EC_{50}	S.D.
UG037.8	0.83	0.014
HT593.1	0.25	0.01
MW965.26	0.55	0.022
BR019.10	0.77	0.11
UG024.2	0.97	0.044
ZR001.3	1.07	0.013
UG975.10	0.87	0.034
Bal-L	0.53	0.002
R2	0.13	0.007
89.6	1.17	0.007
CM243	116.13	1.43
IIIB[a]	0.48	0.002

The K_d values (nM) (approximately equal to EC_{50}) of m18 for gp140 from primary isolates were obtained as described in Fig. 2.
[a] gp120.

Table 2
Inhibition of HIV-1 Env-mediated fusion by m18, Fab b12 and Fab X5

Env/Ab	RW020.5	US715.6	HT593.1	US005.11	89.6	IIIB	NL43	TH022.4	UG975.10
Clade	A	B	B	B	B	B	B	EA	G
m18	83	79	40	87	93	84	63	79	61
X5	89	89	45	89	100	nd	63	87	57
b12	83	96	76	100	100	99	100	86	44

10^5 293 cells, transfected with plasmids encoding various HIV Envs under the control of T7 promoter and infected with recombinant vaccinia virus encoding T7 polymerase gene, were preincubated with m18, X5 or Fab b12 at 200 μg/ml for 30 min at 37 °C, and then mixed with 10^5 CEM-CCR5 cells infected with recombinant vaccinia virus encoding β-galactosidase gene. The extent of cell fusion was quantified colorimetrically 2 h after mixing the cells. The data are averages of duplicate samples and presented as percentage of fusion inhibition. nd means not done.

Table 3
Neutralization of HIV-1 group M Envs by m18 and Fab X5

Clade	HIV-1 isolate	X5	m18
A	92RW020.5	64[a]	100
B	R2[b]	3.6	1.6
	MN-TCLA	12	<1.6
	MN-P	4.4	100
	MACS#6	≥100[b]	>100
	MACS#8	1.9	5.0
	MACS#4	2.8	6.3
	MACS#9	100	≥100
	VI1399	3.9	>100
	VI1273	6.2	≥100
	VI423	12	4.5
C	GX-C44	16	>100 (62?)
D	Z2Z6	5.0	≥100
E	GX-E14	>100	>100 (45)
	TH966	≥100	≥100
F	VI14004[c]	>100	20.0

Neutralization assays were carried out in triplicate wells by preincubation of serial dilutions of Fab m18 and X5 with pseudotype viruses for 1 h at 4 °C followed by infection of 1–2×10^4 HOS $CD4^+$ $CCR5^+$ ($CXCR4^+$) cells. Luminescence was measured after 3 days. The mean luminescence readings for triplicate wells were determined, and the endpoint was considered to be the last dilution of Fabs at which the mean results from the test samples were less than 50% of the nonneutralized control mean. The Fab concentration that resulted in 90% neutralization was two to eight (usually four) fold greater than that which produces 50% neutralization. Neutralization assays for each envelope clone against the antibody were carried out two or three times and the averages are shown. The numbers in parentheses denote percent neutralization at 100 μg/ml.

[a] Concentration of the mAb Fab at which 50% neutralization of the test samples were observed when compared to non-neutralized controls. Representative results of experiments performed at two or three times are shown.

[b] IC_{50} for at least one of the experiments was less or equal to 100 μg/ml.

[c] Patients whose sera demonstrated broad cross-neutralizing activity.

et al., submitted). These results suggest that m18 can neutralize a broad range of HIV-1 primary isolates.

4. Discussion

Identification of novel broadly cross-reactive human monoclonal antibodies (mAbs) to various antigens has major implications for development of vaccines, inhibitors and tools to study mechanisms. Perhaps the most important practical implication of the identification of such antibodies is the possibility that their conserved epitopes could serve as immunogens for elicitation of protective immunity against viruses that show high degree of genetic polymorphism. Here we have described an experimental strategy that could facilitate the selection of broadly cross-reactive antibodies, and used it for selection of a novel human mAb (m18) against the gp120 subunit of the Env. This mAb bound with high affinity to different Envs, and exhibited broad HIV neutralizing activity.

Of the large number of mAbs and Fabs that have been generated against HIV envelope glycoproteins (Envs) until recently, only three human mAbs were identified that exhibit broad and potent HIV neutralizing activity (D'Souza et al., 1997): two against gp120-IgG1 b12 (Burton et al., 1994; Roben et al., 1994) and 2G12 (Sanders et al., 2002; Scanlan et al., 2002; Trkola et al., 1996), and one against gp41, 2F5 (Muster et al., 1993). Two other antibodies against gp120, the Fab X5 (Moulard et al., 2002) and IgG 447-52D and two against gp41, 4E10 (Stiegler et al., 2001; Zwick et al., 2001b) and Fab Z13 (Zwick et al., 2001b) are also known to neutralize a variety of HIV-1 primary isolates from different clades. The identifi-

cation of a new broadly cross-reactive HIV neutralizing mAb Fab suggests that such antibodies could play even more important role in vivo than anticipated. A note of caution is that the Fab of this antibody was tested but not the whole antibody that could have properties different from those of the Fab; such molecule is being generated. In addition, we have no evidence yet that m18 does exist in patients and plays any role in virus neutralization in vivo.

The SAP methodology that was used here to facilitate the identification of novel broadly cross-reactive HIV neutralizing antibodies could be also used for any antigen that shares common epitopes with other antigens. Examples include but are not limited to rapidly mutating viruses and cancer cells, as well as proteins that share common structural elements. Finally, variations of this methodology can be devised including strategies to use more antigens in different order during panning and screening.

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References

Barbas, C.F., Burton, D.R., Scott, J.K., Silverman, G.J., 2001. Phage Display: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Beirnaert, E., Nyambi, P., Willems, B., Heyndrickx, L., Colebunders, R., Janssens, W., van der, G.G., 2000. Identification and characterization of sera from HIV-infected individuals with broad cross-neutralizing activity against group M (env clade A–H) and group O primary HIV-1 isolates. J. Med. Virol. 62, 14–24.

Burton, D.R., 2002. Antibodies, viruses and vaccines. Nat. Rev. Immunol. 2, 706–713.

Burton, D.R., Pyati, J., Koduri, R., Sharp, S.J., Thornton, G.B., Parren, P.W., Sawyer, L.S., Hendry, R.M., Dunlop, N., Nara, P.L., et al., 1994. Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. Science 266, 1024–1027.

Dimitrov, D.S., Golding, H., Blumenthal, R., 1991. Initial stages of HIV-1 envelope glycoprotein-mediated cell fusion monitored by a new assay based on redistribution of fluorescent dyes. AIDS Res. Hum. Retrovir. 7, 799–805.

D'Souza, M.P., Livnat, D., Bradac, J.A., Bridges, S.H., 1997. Evaluation of monoclonal antibodies to human immunodeficiency virus type 1 primary isolates by neutralization assays: performance criteria for selecting candidate antibodies for clinical trials. AIDS clinical trials group antibody selection working group. J. Infect. Dis. 175, 1056–1062.

Moulard, M., Phogat, S.K., Shu, Y., Labrijn, A.F., Xiao, X., Binley, J.M., Zhang, M.Y., Sidorov, I.A., Broder, C.C., Robinson, J., Parren, P.W., Burton, D.R., Dimitrov, D.S., 2002. Broadly cross-reactive HIV-1-neutralizing human monoclonal Fab selected for binding to gp120–CD4–CCR5 complexes. Proc. Natl. Acad. Sci. U. S. A. 99, 6913–6918.

Muster, T., Steindl, F., Purtscher, M., Trkola, A., Klima, A., Himmler, G., Ruker, F., Katinger, H., 1993. A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1. J. Virol. 67, 6642–6647.

Nussbaum, O., Broder, C.C., Berger, E.A., 1994. Fusogenic mechanisms of enveloped-virus glycoproteins analyzed by a novel recombinant vaccinia virus-based assay quantitating cell fusion-dependent reporter gene activation. J. Virol. 68, 5411–5422.

Park, E.J., Vujcic, L.K., Anand, R., Theodore, T.S., Quinnan Jr., G.V., 1998. Mutations in both gp120 and gp41 are responsible for the broad neutralization resistance of variant human immunodeficiency virus type 1 MN to antibodies directed at V3 and non-V3 epitopes. J. Virol. 72, 7099–7107.

Quinnan Jr., G.V., Zhang, P.F., Fu, D.W., Dong, M., Margolick, J.B., 1998. Evolution of neutralizing antibody response against HIV type 1 virions and pseudovirions in multicenter AIDS cohort study participants. AIDS Res. Hum. Retrovir. 14, 939–949.

Quinnan, G.V., Zhang, P.F., Fu, D.W., Dong, M., Alter, H.J., 1999. Expression and characterization of HIV type 1 envelope protein associated with a broadly reactive neutralizing antibody response. AIDS Res. Hum. Retrovir. 15, 561–570.

Roben, P., Moore, J.P., Thali, M., Sodroski, J., Barbas III, C.F., Burton, D.R., 1994. Recognition properties of a panel of human recombinant Fab fragments to the CD4 binding site of gp120 that show differing abilities to neutralize human immunodeficiency virus type 1. J. Virol. 68, 4821–4828.

Sanders, R.W., Venturi, M., Schiffner, L., Kalyanaraman, R., Katinger, H., Lloyd, K.O., Kwong, P.D., Moore, J.P., 2002. The mannose-dependent epitope for neutralizing antibody 2G12 on human immunodeficiency virus type 1 glycoprotein gp120. J. Virol. 76, 7293–7305.

Scanlan, C.N., Pantophlet, R., Wormald, M.R., Ollmann, S.E., Stanfield, R., Wilson, I.A., Katinger, H., Dwek, R.A., Rudd, P.M., Burton, D.R., 2002. The broadly neutralizing anti-human immunodeficiency virus type 1 antibody 2G12 recognizes a cluster of alpha1->2 mannose residues on the outer face of gp120. J. Virol. 76, 7306–7321.

Stiegler, G., Kunert, R., Purtscher, M., Wolbank, S., Voglauer, R., Steindl, F., Katinger, H., 2001. A potent cross-clade neutralizing human monoclonal antibody against a novel epitope on gp41 of human immunodeficiency virus type 1. AIDS Res. Hum. Retrovir. 17, 1757–1765.

Trkola, A., Purtscher, M., Muster, T., Ballaun, C., Buchacher, A., Sullivan, N., Srinivasan, K., Sodroski, J., Moore, J.P., Katinger, H., 1996. Human monoclonal antibody 2G12 defines a distinctive neutralization epitope on the gp120 glycoprotein of human immunodeficiency virus type 1. J. Virol. 70, 1100–1108.

Zwick, M.B., Bonnycastle, L.L., Menendez, A., Irving, M.B., Barbas III, C.F., Parren, P.W., Burton, D.R., Scott, J.K., 2001a. Identification and characterization of a peptide that specifically binds the human, broadly neutralizing anti-human immunodeficiency virus type 1 antibody b12. J. Virol. 75, 6692–6699.

Zwick, M.B., Labrijn, A.F., Wang, M., Spenlehauer, C., Saphire, E.O., Binley, J.M., Moore, J.P., Stiegler, G., Katinger, H., Burton, D.R., Parren, P.W., 2001b. Broadly neutralizing antibodies targeted to the membrane-proximal external region of human immunodeficiency virus type 1 glycoprotein gp41. J. Virol. 75, 10892–10905.